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THE ANATOMY OF OPHIOGLOSSUM PENDULUM

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(WITH SIXTEEN FIGURES)

The section OPHIODERMA of the genus *Ophioglossum*, as first separated by PRANTL (6), is distinguished from EUOPHIOGLOSSUM by the numerous vascular strands in the base of the petiole, and from CHEIROGLOSSA, which shares this feature, by the occurrence of a single spike on each fertile leaf. As originally described, the section was composed of the single species *O. pendulum* L.; *O. intermedium* Hook. was considered to be a young form of *O. pendulum*, and was included with that species. In 1904, BOWER (2) described the new species *O. simplex* Ridley, from Sumatra, and from an examination of its vascular system showed that it is a member of this section. He further reestablished the species *O. intermedium* Hook. on the basis of its intermediate position between *O. pendulum* and *O. simplex*.

Of the three species constituting the section, *O. pendulum* is best known. BOWER (1) has described the development of the fertile spike and sporangia, and in a later paper (2) has given a brief account of its anatomy. This paper deals merely with the vascular system of the leaf and its insertion upon that of the stem. The investigation here described has been undertaken with a view to supplementing this account of the anatomy of the species.

Material

A large number of entire plants was collected by Dr. CHARLES J. CHAMBERLAIN in November 1911, in Northern Australia. The exact locality is near Babinda, in the Cairns district of Queensland, at a distance of about 25 miles from the coast. Occasional clusters of plants were found near the ground, but more usually they occurred at a considerable height, up to 30 meters. In almost all cases they were growing in the masses of humus accumulated by large plants of *Polypodium rigidulum; Lycopodium phlegmaria* was a common associate. Plants of this species were also observed on Stradbroke Island, near Brisbane, but no collections were made. The groups of plants here were at a less distance from the ground; they were commonly attached to plants of *Platycerium alcicorne* and *P. grande*.

In the material secured at Babinda, the largest leaves had attained a length of about 1.5 meters. However, some of the plants on Stradbroke Island had leaves nearly 2 meters long, and Mr. WM. GIBSON, a collector familiar with that region, reported a specimen with leaves measuring 2.7 meters in length. The largest spikes seen measured 30 cm. in length and 1.2 cm. in greatest width. Many cases of branching or lobing of the spikes were observed; probably one-tenth of the specimens showed some variation from the simple form.

This supply of entire plants was supplemented by a large number of rhizomes, root tips, buds, and spikes collected by Dr. W. J. G. LAND on the island of Tutuila, Samoa, in October 1912. These plants, together with many ferns and occasional plants of *Lycopodium phlegmaria*, occurred in the root masses of *Asplenium nidus*, at a height of 2–10 meters; a large cluster is shown in fig. 1. The leaves reached a length of 2 meters; branching of the leaf was not uncommon, but branching or lobing of the spike was relatively rare. The largest spike collected measured 51 cm. in length and 1.3 cm. in width; sporangia occurred along 45 cm. of its length. Several spikes more than 40 cm. in length were found.

The Australian material was preserved in 6 per cent formalin; that secured in Samoa was killed in 50 per cent alcohol and 6 per cent formalin. Even the largest rhizomes cut readily in paraffin at 10–25 μ, and complete series were easily obtained. The safranin-anilin blue stain combination gave the best results.

FIG. 1.—*Ophioglossum pendulum*, epiphytic upon *Citrus* sp.; Tutuila, Samoa; photograph by Dr. W. J. G. LAND.

The root

The root stele at the point of attachment to the stem cylinder varies from diarch to pentarch; triarch and tetarch arrangements of the xylem are commonest. In passing from the base of the root toward the tip, the number of protoxylem points frequently increases by the splitting of one of the original protoxylem strands; in one case, the disappearance of a protoxylem strand was noted. In a few cases only, a hexarch arrangement was found; these roots were all very large. In general there is a definite relation between the size of the root and the number of protoxylem strands.

Each phloem mass is a layer 1 or 2 cells in thickness, separated from the xylem by 3–5 layers of parenchyma. It is easily distinguished by the somewhat thickened walls and the conspicuous "proteid granules" adhering to the walls. In the apical region of the root, the protophloem can usually be distinguished considerably in advance of the protoxylem. When a protoxylem strand splits, the phloem between the branches of the split strand connects with the phloem masses on either side.

There is no pericyclic layer; the phloem abuts directly upon the endodermis (fig. 10), which can be readily distinguished by the suberized band upon the radial walls; it resembles the endodermis of *Botrychium virginianum* in all particulars. Two or three cells of parenchyma separate the protoxylem from the endodermis.

Two general regions of the cortex about equal in thickness are distinguishable. In the inner region, the cell walls are heavily thickened by cellulose; simple circular or oval pits occur. In the outer region, the thickening of the cell walls is much less and gives the reactions of pectin compounds. Intercellular spaces of considerable size occur in the inner region; in the outer region, these are much smaller and become filled by the substance which constitutes the thickening of the cell walls. The endophytic fungus which occurs commonly in the roots is confined to the outer region.

The outer walls of the surface cells are remarkably thickened, as shown in fig. 2. The thickening material is not cellulose, but gives the reaction of pectin compounds. The thickening may continue until the lumen of the cell is almost filled; even in these conditions the protoplasm remains very active. When cells of the epidermal layer are killed, the outer walls of the cells immediately below begin to thicken in this way. This may occur in any of the cells of the outer region of the cortex upon destruction of the overlying cells.

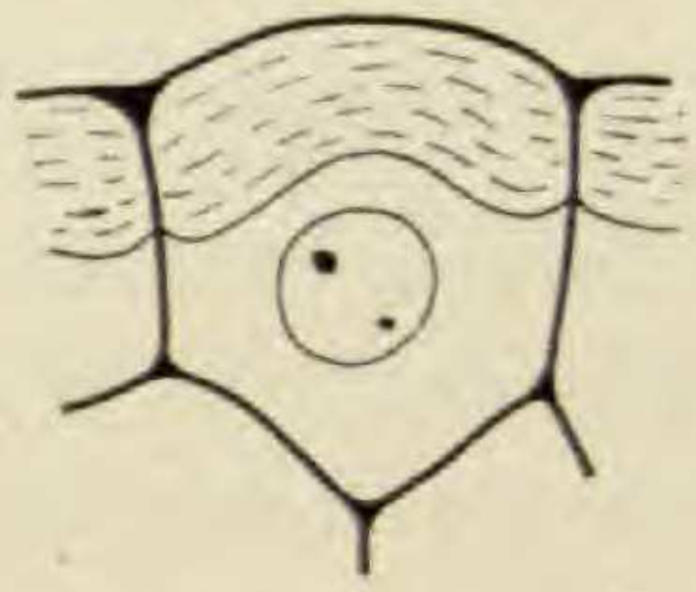

FIG. 2.—Epidermal cell of root; ×236.

Roots originate in the meristematic region of the stem by the formation of an apical cell in the first layer of cells outside the phloem. Fig. 3 represents this origin as seen in a longitudinal section of the stem. As shown, differentiation of the phloem has proceeded to within a few cells of the position of the root meristem. The growth of the root proceeds by the segmentation of the apical cell, which is tetrahedral in form; in slowly growing roots the segmentation is irregular, and a definite root cap cannot be distinguished. The first xylem elements are smaller in cross-section than those formed later; they are true tracheids with walls reticulately thickened. In a few cases of more rapidly growing roots, the thickening of the walls approaches the annular marking. This character of the protoxylem indicates the slow growth of the roots. The lignification proceeds slowly toward the center of the stele, and xylem parenchyma is often found in the center of the stele of large roots at a distance of 3–4 cm. from the tips. There is no secondary thickening of the xylem mass. In a few cases a medullated

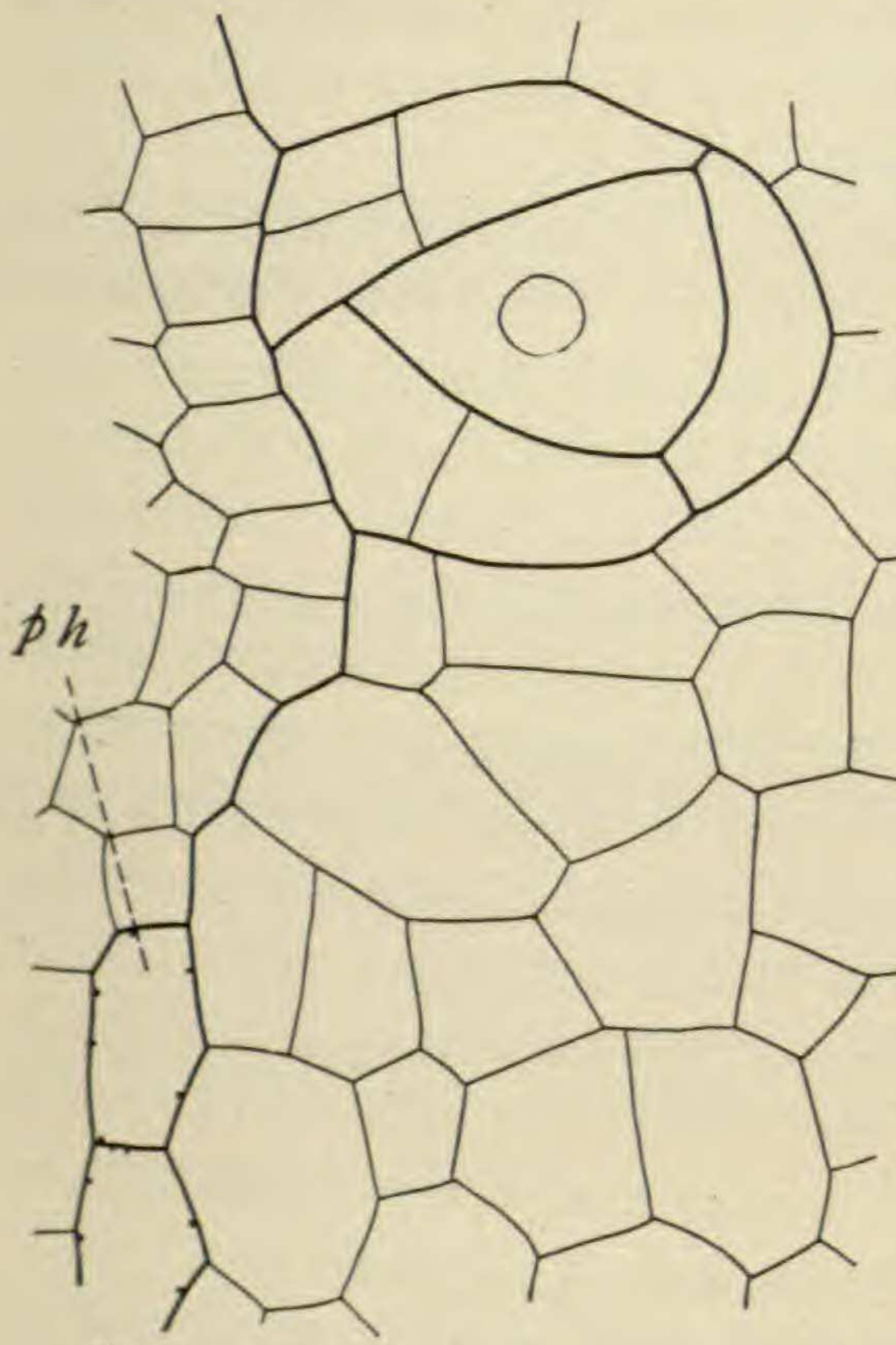

FIG. 3.—Origin of root, as shown in longitudinal section of a bud: *ph*, phloem; ×236.

condition occurred, as shown in fig. 4; the pith is not xylem parenchyma, but is composed of cells resembling those of the cortex; the opening through the xylem always occurs, but the endodermis is not interrupted. This occurs only in roots upon which buds have developed stems; the open portion of the xylem mass is upon the upper side of the root. This condition persists for only a short distance from the bud toward the tip of the root; the gap soon closes and a solid xylem mass is again formed. This peculiar root stele form is directly related to the formation of the stem stele from buds upon the roots, as described later.

The branching of the root is strictly monopodial. The lateral roots grow at an angle of 60–90° to the main root; two or three branch roots often develop at one point. These are not restricted in their growth, as in *Helminthostachys*, but may reach as great length as the primary roots.

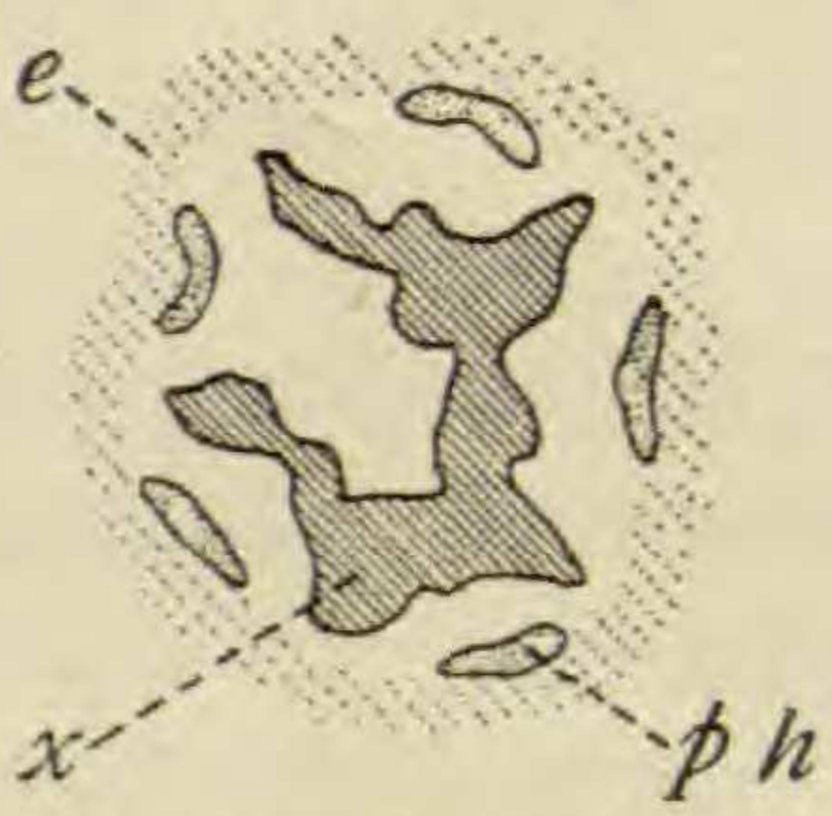

FIG. 4.—Medullated root stele: *x*, xylem; *ph*, phloem; *e*, endodermis; ×37.

Immediately before a branch is given off, the phloem of the main stele spreads laterally between the endodermis and the protoxylem, and forms a continuous sheath about the xylem. A strand of xylem from each of two poles of the main root runs into the branch; hence the lateral roots at the point of attachment are diarch. The phloem sheath surrounds the xylem of the branch in the same way that it does that of the main root; the endodermis also connects without a gap. After the branch has separated, the phloem between the protoxylem and the endodermis disappears, and the usual arrangement of the stelar elements is restored.

In all cases, the branch stele is diarch at the point of attachment, but it may arise from a single pole of the main root. The protoxylem strands of the branch soon split, and the usual triarch or tetrarch condition is established. In one case, a branch arises from a large tetrarch root in which lignification has just begun; each pole is represented by three or four tracheids only. Two distinct strands of xylem, connecting with two poles of the main root, enter the branch. Each xylem strand is surrounded by a sheath of phloem and endodermis. At a little distance, the endodermal sheaths come into contact, then fuse to form a single sheath; the phloem shows the same behavior. Xylem parenchyma then appears between the two protoxylem strands and the usual diarch condition of the branch stele is established.

The bud

The vegetative reproduction of the species is accomplished by buds upon the roots, as in *O. vulgatum*. As many as three buds were observed upon a single root; the second leaf of the oldest bud was just appearing, while the youngest bud was a mere swelling about 5 mm. from the root tip. It is almost certain that the plants of a colony have all developed from a single plant by this method of vegetative propagation; every rhizome examined in which the base is intact shows by its connection with a root that it has developed from a bud; fig. 5 represents such a case.

FIG. 5.—A rhizome showing radial character: *a*, stem tip; *lb*, base of a decayed leaf; *r*, parent root; ×1.

ROSTOWZEW (7) has described the development of the bud in *O. vulgatum*. In the second segment of the apical cell of the root a new apical cell arises; this produces all stem tissues. The root apical cell is retarded for a time, but finally resumes growth. This results in the formation of a bud with its axis approximately at right angles to the parent root.

The development of the bud has been examined in *O. pendulum;* it agrees in all important points with that of *O. vulgatum*. The retardation of the root growth is usually less; the bud axis often diverges less abruptly from that of the root. Two roots usually develop upon a bud before the first leaf is formed. The details of the vascular connection between the stem developed from a bud and the parent root will be described later.

The stem

The largest rhizome of the Australian material measures 0.7 cm. in diameter and 2.5 cm. in length. There are 18 leaves still attached and the bases of 5 others are evident. All the rhizomes, with the exception of the very youngest, give evidence of having grown in a horizontal position; field observations by Dr. CHAMBERLAIN confirm this. The leaf bases, however, are not restricted to the dorsal side, as stated by CAMPBELL (3) for this species, but are attached in a spiral about the stem. The distribution is irregular; on young stems the leaves are rather crowded and attached along a spiral with about a $\frac{1}{3}$ arrangement. On older stems the distribution approximates a $\frac{3}{8}$ arrangement; fig. 6 shows the arrangement of the leaves on the largest specimen secured. The bases of the leaves inserted on the lower side curve round the rhizome and produce the appearance of a 2-ranked arrangement, as in *Helminthostachys;* but there is nothing in the insertion of the leaves or the structure of the stem to indicate true dorsiventrality.

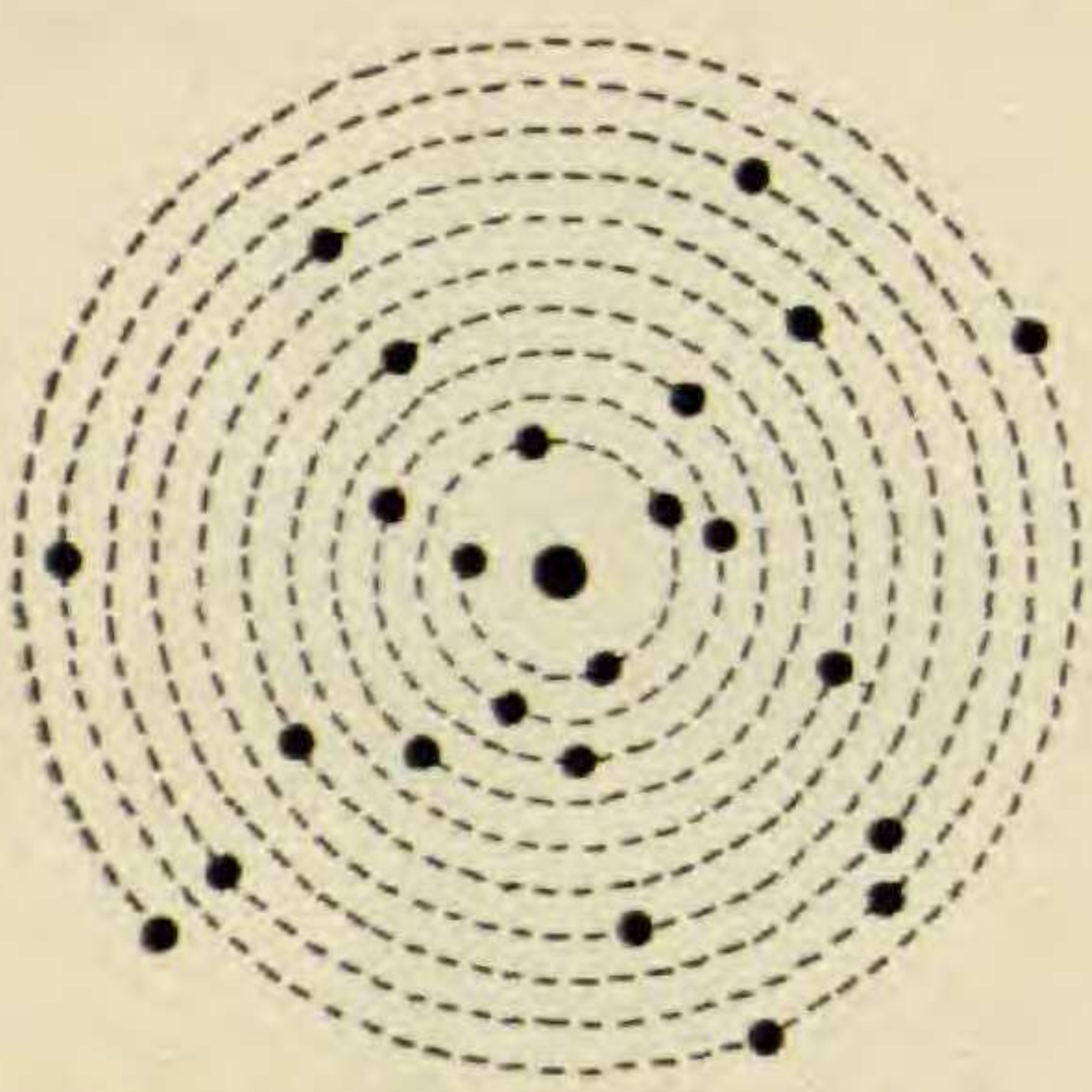

FIG. 6.—Diagram of leaf arrangement of a horizontally growing rhizome.

The rhizomes of the Samoan material are decidedly larger than those from Australia; the oldest one secured, with 8 functional leaves and 7 leaf bases, was 1.2 cm. in diameter and 4.6 cm. in length. Both their appearance and their structure indicate a definite radial arrangement (fig. 5). The leaf insertion is similar to that of the Australian specimens, but the leaves are less crowded.

All the rhizomes in which the bases are intact give evidence of having developed from buds upon roots in the manner described above. The connection of the stem stele with that of the root was examined in 12 specimens; five methods of development of the stem stele were found.

In a single specimen, a solid strand of xylem, surrounded by phloem and endodermis, separates at a considerable angle from one of the protoxylem strands of a tetrarch root (fig. 7). The endodermis soon disappears; before separation from the root stele is complete, a few cells of parenchyma appear near the center of the xylem mass (fig. 7, *C*). The number of these increases and a definite pith is established (fig. 7, *D*). A small gap opens through the xylem, and closes again almost at once; the phloem is not interrupted. The xylem cylinder dilates rapidly and becomes oval in section; root steles attach at the points of the oval, leaving large "root gaps" in the cylinder (fig. 7, *L*). A third root attaches between the two gaps (fig. 7, *M*), producing a small gap; the three gaps close at about the same level. The first strand of the trace

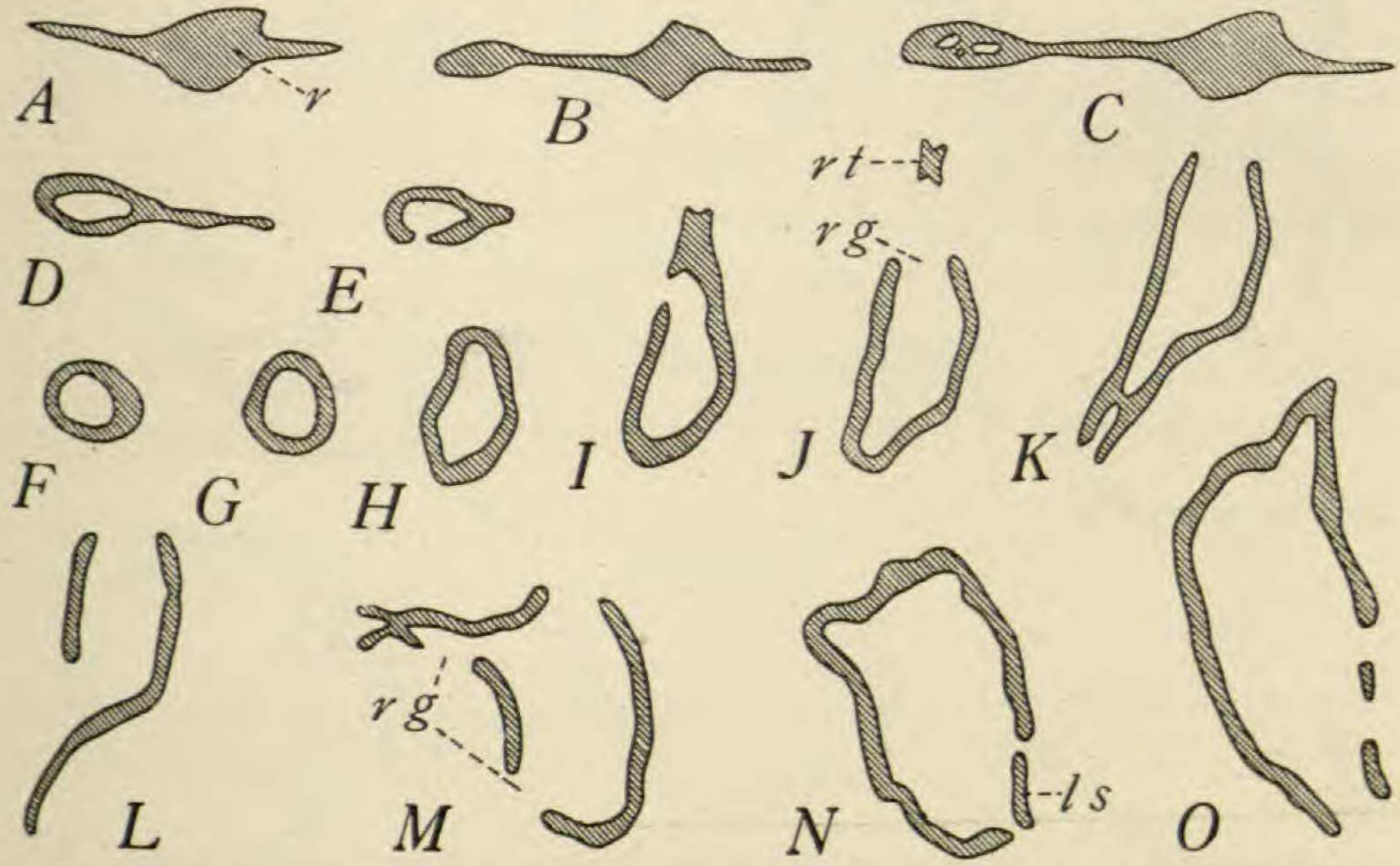

FIG. 7.—Development of stem stele from a single strand; only the xylem is shown: *r*, tetrarch stele of parent root; *rt*, root trace; *rg*, root gap; *ls*, first strand of first leaf; ×12.

of the first leaf separates from the cylinder at a slightly higher level (fig. 7, *N*). This is essentially the course of development of the stem stele of *O. vulgatum* from a bud.

In five cases, two strands of xylem of varying shape in cross-section separate from two of the poles of a triarch or tetrarch root. Diagrams of sections of one of these specimens are given in fig. 8. Each strand at the point of junction with the root stele has phloem on two faces, or surrounding it; the phloem disappears almost immediately from the adjacent faces and becomes restricted to the exterior sides of the strands. After an interval the two strands fuse at one margin, forming a half-cylinder of xylem (fig. 8, *E*). Two roots are usually given off somewhat farther up (fig. 8, *I*), and the margins of the gap come together at about the level of the base of the first leaf.

In a single instance, three strands arise from the three poles of a triarch root. These fuse just below the point of connection of the first root to form a large strand of semicircular cross-section. The gap between the margins closes just below the level of the first leaf. In another specimen, two strands separate from each of two of the

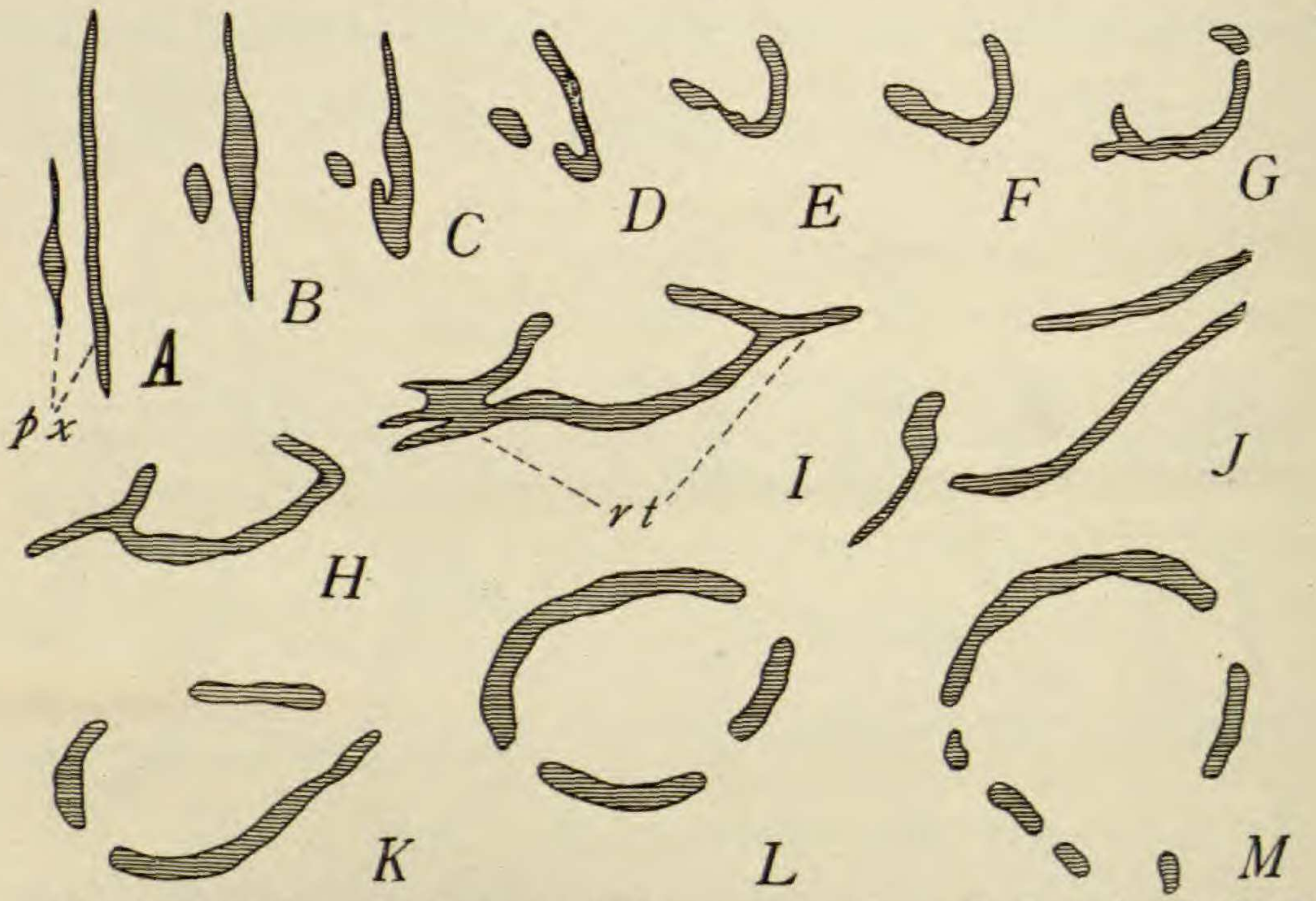

FIG. 8.—Development of stem stele from two strands; only the xylem is shown: *px*, protoxylem strands of parent root; *rt*, root traces; ×12.

protoxylem strands of a tetrarch root. These four organize a stele in the manner described above; the opening in the side is closed rather late, at the level of the top of the first leaf gap.

The stem stele is organized in a distinctly different way in four cases. A series of sections through one of the specimens from below upward is shown by fig. 9. In this case, the xylem mass of one pole of a diarch root begins to enlarge (fig. 9, *A–C*). After a time, medullation of the enlarged xylem strand occurs by the appearance of parenchyma near the center (fig. 9, *D*, *E*, fig. 10). The endodermis disappears on the side away from the unchanged pole of the root. At the same level, gaps appear in the sides of the cylinder (fig. 9, *G*, *H*,) and the xylem separates into two strands. The smaller of these (*r*, fig. 9, *H*) is the continuation of the original root, and at once resumes its original diarch character. The larger strand organizes itself into the stem stele. The three strands of the first leaf separate (fig. 9, *I*, *J*), and at a slightly higher level (fig. 9, *J*, *K*) two roots are attached. The stem stele at this point

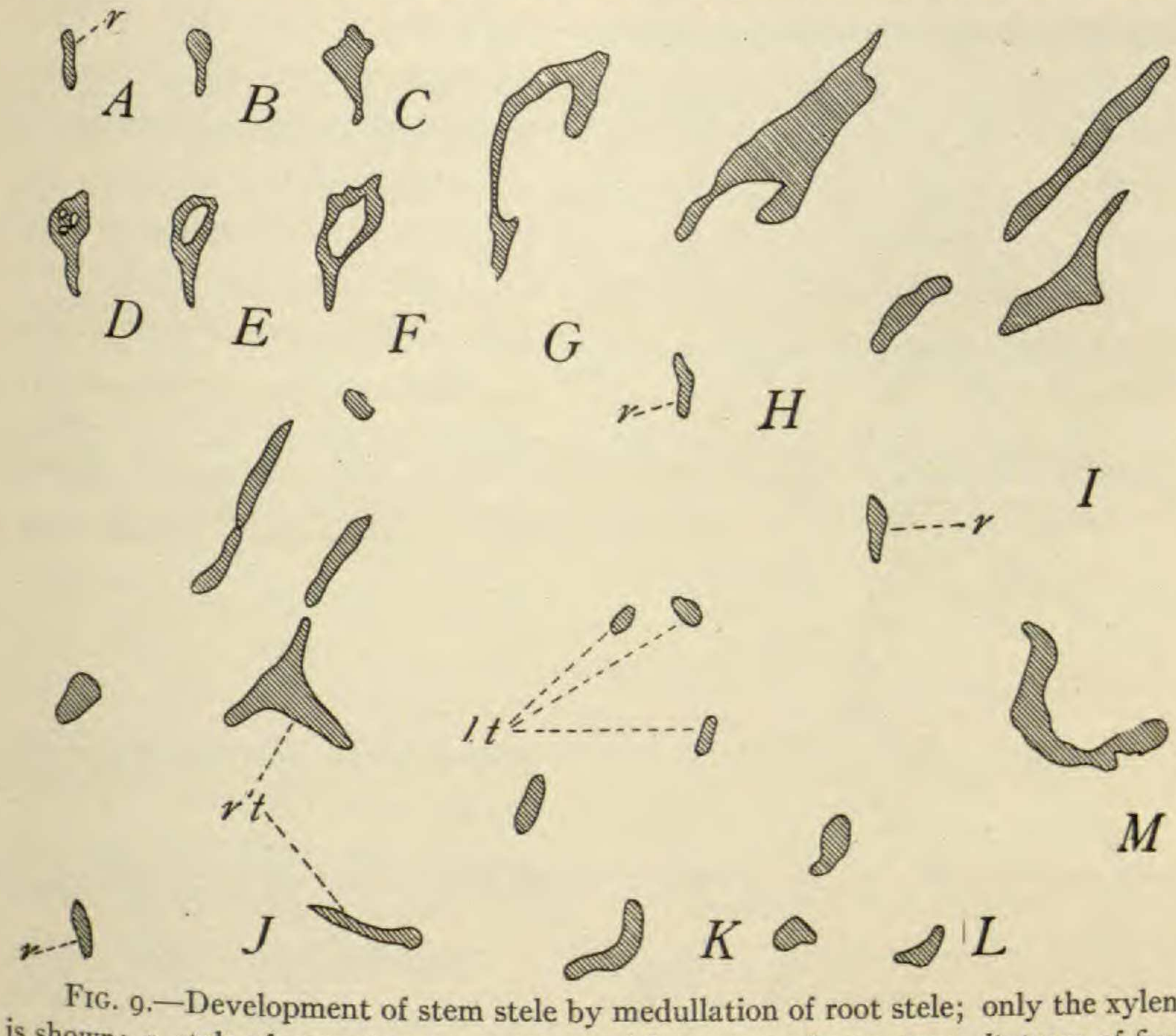

FIG. 9.—Development of stem stele by medullation of root stele; only the xylem is shown: *r*, stele of parent root; *rt*, traces of first roots of new stem; *lt*, trace of first leaf; ×25.

consists of three strands (fig. 9, *L*), which organize the mature form of the stele in the usual way. In the other rhizomes showing this manner of development, the original root is triarch or tetrarch; after separation from the stem stele, the root stele usually shows an increased number of protoxylem strands and the medullated condition shown in fig. 4.

In all cases, the stem stele soon assumes its usual form, that of an ectophloic siphonostele with more or less overlapping leaf gaps (fig. 11). Two gaps usually occur in the same transverse section, and cause the xylem of the stem to appear as two curved masses with concave sides facing (fig. 11, *G*). Often only a single gap interrupts the cylinder (fig. 11, *A*), or the section may show a complete ring of xylem (fig. 12, *C*); very rarely, three gaps overlap. The leaf gaps are circular or oval and usually very large; 2 mm. in width by 2.5 mm. in height is an average size. The largest observed measures 3.2×3.5 mm. In general the stele is more compact and shows more definitely its cylindrical character than in *O. vulgatum*. It is often very irregular in outline; the insertion of leaf and root strands usually produces a modification of shape, as shown in figs. 11, *I;* 12, *E;* 7, *J*, *K*, etc. It is usually not straight, but slightly bent at each leaf gap in the direction away from the leaf; this is undoubtedly due to the pressure of the young leaf. In diameter the stele is usually a little less than half that of the rhizome, that is, 3–5 mm.

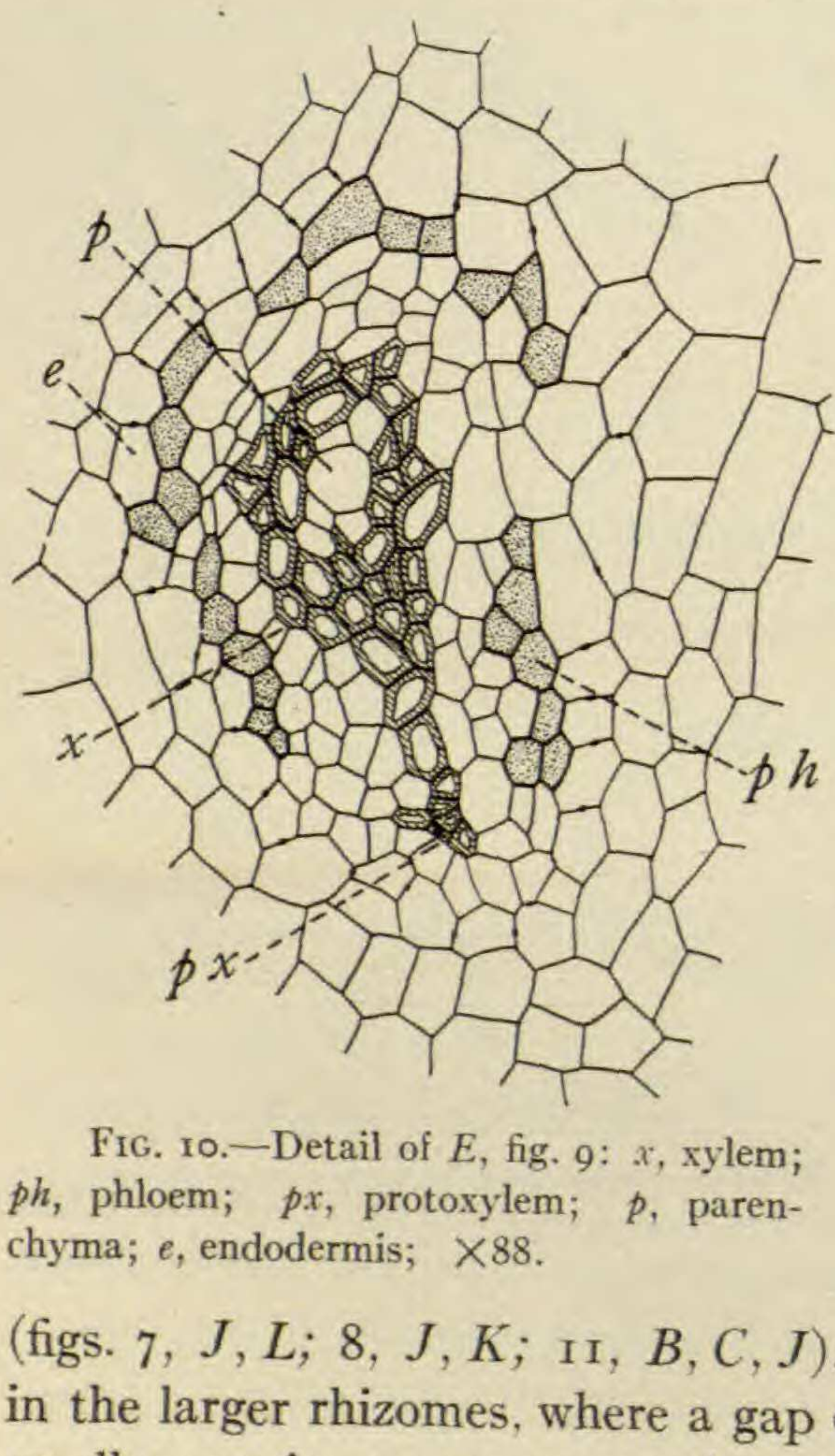

FIG. 10.—Detail of *E*, fig. 9: *x*, xylem; *ph*, phloem; *px*, protoxylem; *p*, parenchyma; *e*, endodermis; ×88.

Besides these leaf gaps, openings definitely related to roots occur in the cylinder (figs. 7, *J*, *L;* 8, *J*, *K;* 11, *B*, *C*, *J*). These are more conspicuous in the larger rhizomes, where a gap occurs above each root; in the smaller specimens, gaps occur in connection with about half of the roots. These openings are narrower in proportion to their length than are the leaf gaps; they range in size from 0.2×0.4 mm. to 0.5×2 mm. Where a root is attached to the cylinder immediately below the point of insertion of a leaf, a gap is almost invariably produced; the leaf strands attach to the sides of this gap, which is therefore continuous with the leaf gap. In cases of this kind, the gap in the cylinder above the insertion of a root might be interpreted as the beginning of the leaf gap; but in very many cases openings of an exactly similar nature occur when only a root is involved. These gaps close in the same manner as leaf gaps; for convenience they may be referred to as root gaps.

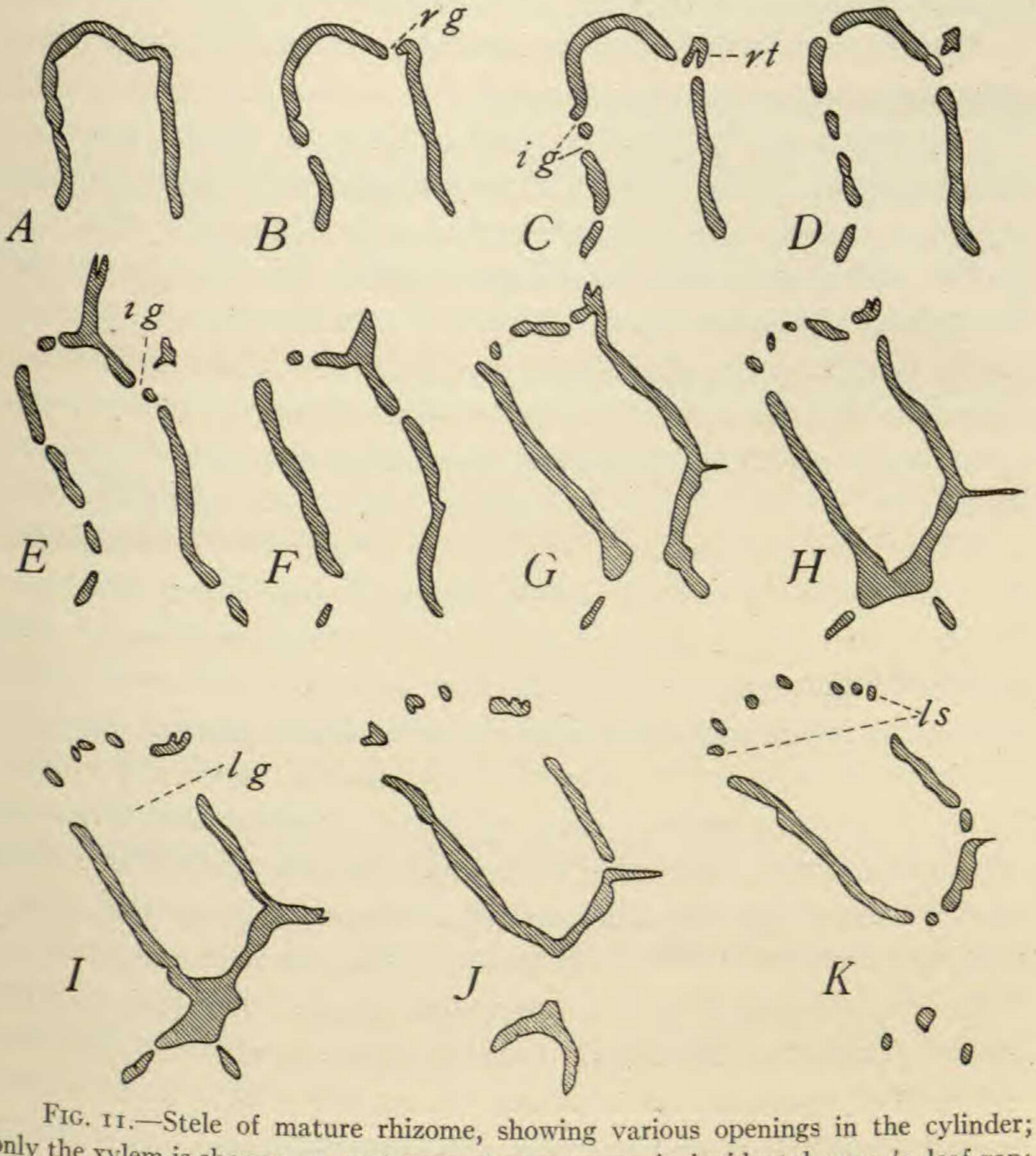

FIG. 11.—Stele of mature rhizome, showing various openings in the cylinder; only the xylem is shown: *rg*, root gap; *rt*, root trace; *ig*, incidental gap; *lg*, leaf gap; *ls*, leaf strands; ×4.5.

In addition to these, openings not related to outgoing strands often occur in the cylinder (fig. 11, *B–F*). These incidental gaps sometimes occur at the margin of a leaf gap; a strand separates from the margin of the gap, runs parallel to it for a time, and later fuses with it. At other times, a long narrow slit may occur in the cylinder. Some of the gaps are relatively large, measuring 0.6×0.7 mm. Apparent gaps, due to a failure of the xylem parenchyma to lignify, sometimes occur; in these the phloem is not interrupted. In most of the incidental gaps, however, the cortical parenchyma connects with that of the pith, as in leaf and root gaps.

Lignification occurs first in the layer of tracheids nearest the pith; these first xylem elements differ in no way from those outside. They are true tracheids, often very irregular in shape; lobed and even branched forms occur. They are relatively short, 3–6 times as long as broad; the walls are reticulately thickened. The lignification does not begin at definite points, but indiscriminately throughout the inner layer. It proceeds in an outward direction; in the mature stems the xylem is 5 or 6 tracheids in thickness. Occasional irregular divisions occur within the procambium strand after lignification has begun, but there is no true secondary thickening.

The phloem is uniformly a single layer of cells; it is separated from the xylem by a layer of parenchyma 3–5 cells in thickness. There is no endodermis except in the extreme basal region and at the points of attachment of roots; in these instances it is a mere extension of the root endodermis, and is not to be considered as related to the stem stele. The phloem abuts directly upon the cortical parenchyma composed of large spherical or ellipsoidal cells with intercellular spaces; the cells of the layers next the phloem are somewhat smaller than those farther out. The walls of all the cortical cells are secondarily thickened with cellulose, as in the inner region of the root cortex; the pits are much larger. The pith is in all respects similar to the cortex; the cells of the layers next the xylem are considerably smaller than the average. There is no starch storage in any part of the plant, but fats occur in some quantity in all parts, especially in the pith and cortex of the stem. The growth of the stem is by a tetrahedral apical cell, as in other species of the genus; its segmentation was not examined.

The largest rhizome of the Australian material presents an interesting variation of this usual situation. The base has decayed and it is impossible to say whether the stem originated from a bud or not. In the lowest part, the cylinder is already definitely organized and of the full diameter. There are five gaps corresponding to leaves that were no longer attached, and 18 leaves were still in position. This is almost certainly the oldest specimen secured.

Up to the level of the fourth leaf gap, the stem cylinder agrees in all details with the usual form, as described above; but about midway of the gap, a strand of xylem separates from the margin of the gap, swings over to the center of the opening, broadens, and by connection with the xylem at either side closes the gap. The gap of the sixth leaf is closed by a similar strand. The gaps of the fifth and seventh leaves are closed in a similar manner by strands which arise as procambium in the parenchyma of the openings. At the level of the fourth leaf, a strand separates from the inner surface of the cylinder opposite the point of connection of a root. This strand runs in the pith near the middle of the cylinder for a distance of about 4.5 mm. and finally closes the gap of the eighth leaf in the manner described. In addition to these five strands, there are in this portion of the stem seven others which arise as procambium or by separation from the inner surface of the cylinder and disappear in the same way, without being in any way related to leaf gaps. One of these closes an incidental gap of the cylinder.

From the level of the eighth leaf to that of the twelfth, there are no medullary strands, and the stele presents the usual appearance. At this point another system of seven strands appears; their positions and behavior are shown in fig. 12. Between the seventeenth and twentieth leaves, no medullary strands occur; but at the level of the latter, three more strands appear as procambium in the pith. One appears in the opening caused by the twenty-first leaf and closes that gap; another appears in the center of the base of the twenty-second leaf, and moves inward and closes the gap. The third, arising near the center of the cylinder, branches once or twice; one branch is recognizable as a procambium strand at a short distance from the apical region.

In all, 23 medullary strands occur: 16 arise as procambium in the pith, 5 as branches from the inner surface of the cylinder, and 2 as branches of other strands; 9 of them are concerned in the closure of leaf gaps, 7 fuse with the inner surface of the cylinder, 4 disappear by fusion with other strands, and 3 disappear by the gradual fading out of the procambium. They range in length from

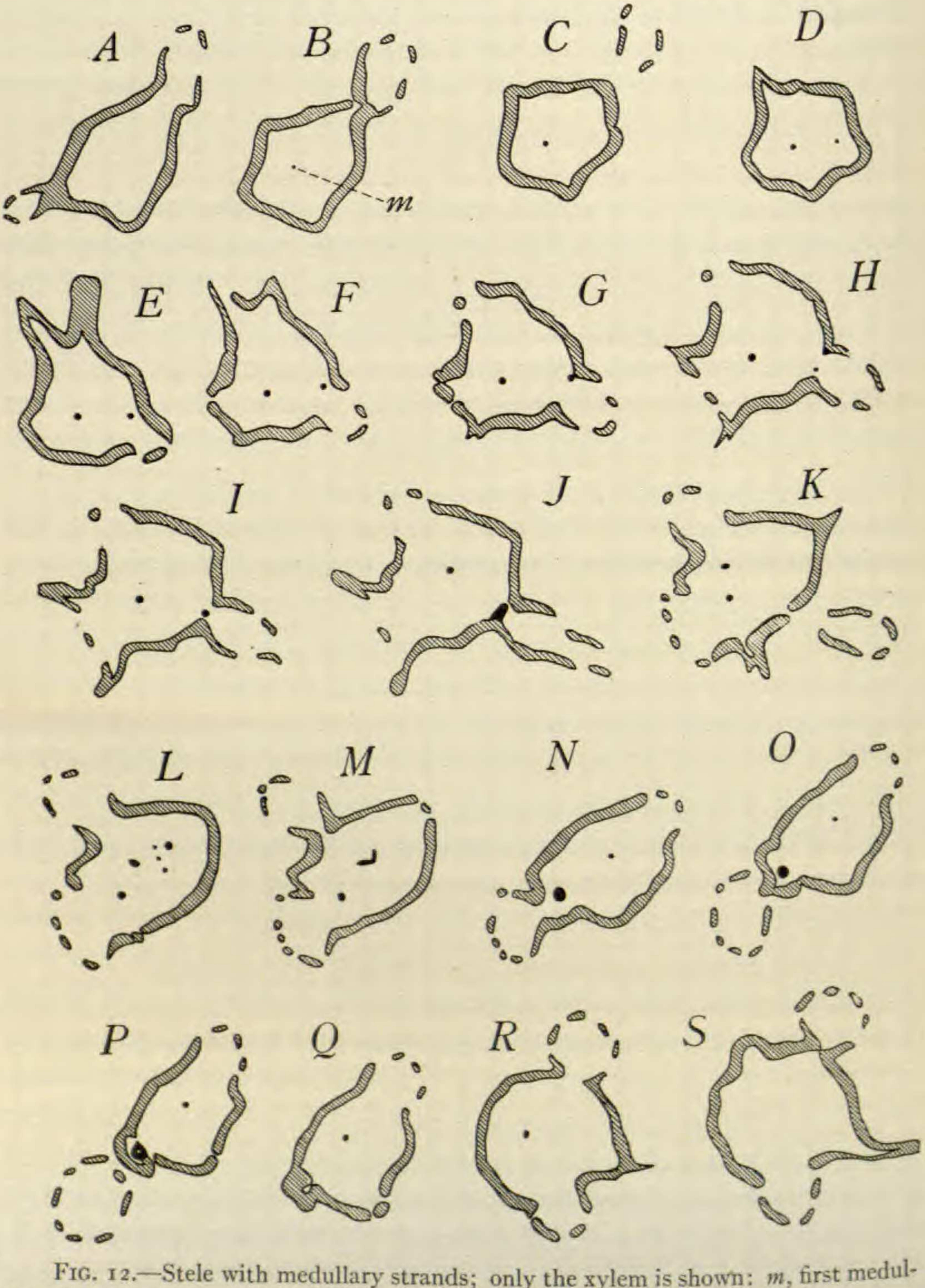

FIG. 12.—Stele with medullary strands; only the xylem is shown: *m*, first medullary strand; ×4.5.

3 or 4 tracheids up to 5.4 mm. in the case of the strand concerned in the closure of the gap of the fifteenth leaf, as shown in fig. 12. A cross-section of one of the strands is shown in fig. 13; they consist of xylem and parenchyma, without a trace of phloem. The largest show 30–40 tracheids in cross-section; 8–12 tracheids is the usual size. No protoxylem can be identified; the tracheids all resemble those of the cylinder.

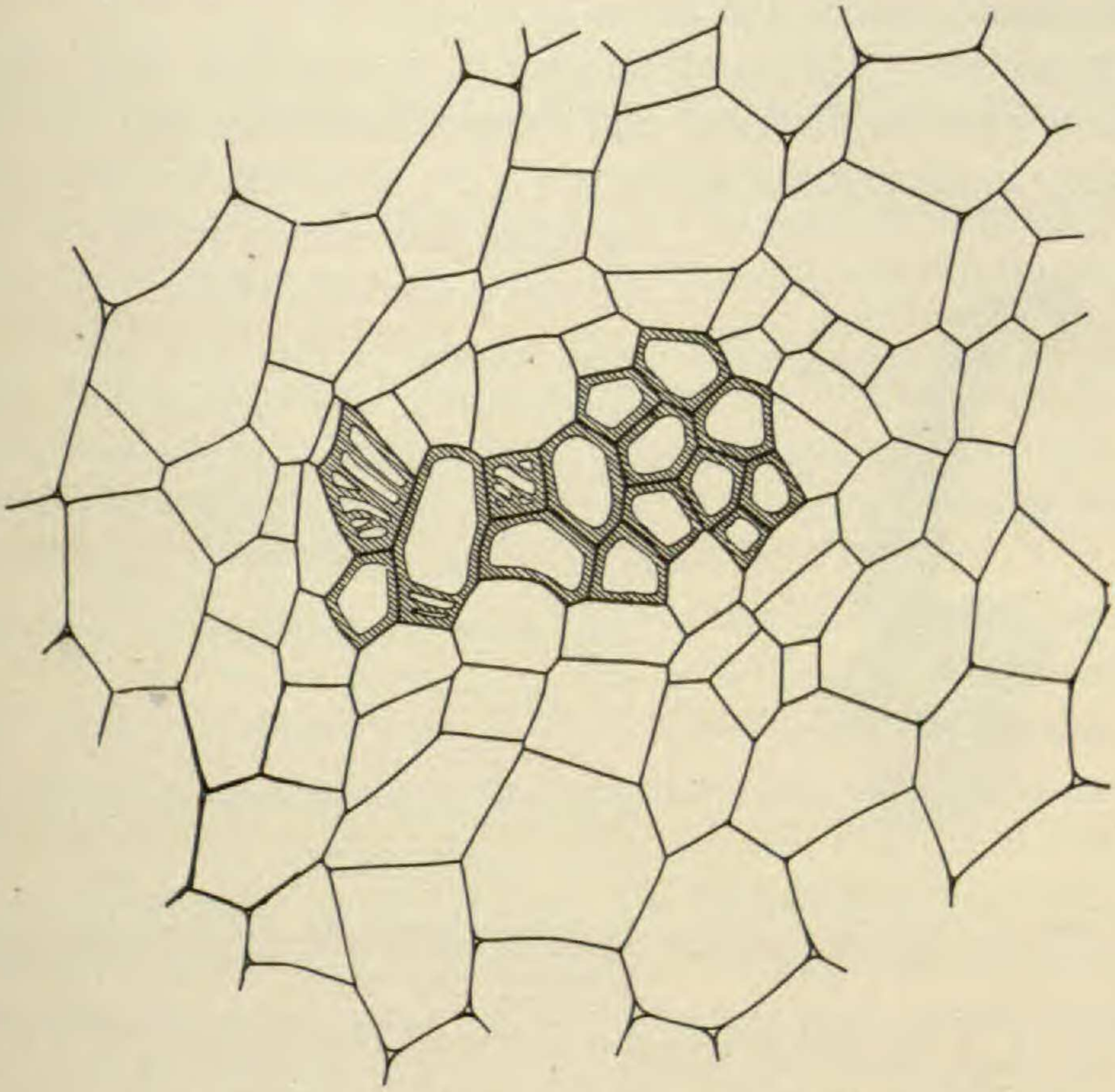

FIG. 13.—Detail of a medullary strand; ×236

In only one other rhizome was anything of this nature observed. This specimen is from Samoa, and has only three leaves; the base is preserved and shows its origin from a bud. At the level of the second leaf a small strand, consisting of a few tracheids only, arises as a procambium at a short distance from the inner surface of the cylinder. It very soon fuses with the cylinder but remains evident as a ridge for a considerable interval. Fron the point of its appearance to that of disappearance is about 0.35 mm.

The leaf

The relation between roots and leaves is very variable. Two or more roots usually appear on the buds before the formation of the first leaf. In the mature stele, roots often connect with the cylinder immediately below a leaf gap; but it is equally common to find two roots attached at the sides of the base of a leaf. No definite relationship between the two can be shown.

As already stated, the leaves in exceptional cases reach a length of 2.7 meters. The stout circular or oval petiole may measure 1 cm. or more in diameter and merges insensibly into the blade; the latter in unbranched leaves is 2–3 cm. in width, but in branched or lobed leaves may reach 5 or 6 cm. at the point of division. Branching and lobing of the leaf occurs very commonly in the Samoan material; in all cases the separation occurs beyond the point of attachment of the fertile spike. There is no absciss layer, as described for *Botrychium virginianum* by JEFFREY (4).

FIG. 14.—Rhizome with young leaf: *s*, fertile spike; ×1.

In the bud, the tip of the leaf is curved over; the fertile spike when first recognizable is attached just beyond the curve, with its tip directed toward the stem. The original development of the leaf is by an apical cell, but its later enlargement is by intercalary growth. As indicated by fig. 14, this begins at the base and proceeds toward the tip. The portion between the spike and the stem may be 4–5 cm. in length when the portion beyond the spike is only 2–3 mm. long. In the mature leaf, the spike is attached at a point about one-third of the way from the base to the tip of the leaf.

The number of strands separating from the cylinder to constitute the leaf trace varies from 3 to 12; 5 is the commonest number. There is a general relation between the size of the leaf, the size of the gap, and the number of strands. The strands usually attach to the cylinder in a circle, the uppermost one after the leaf gap is actually closed (fig. 12, *P;* fig. 11, *I*); in some cases, however, all the strands connect within the lower portion of the opening in the cylinder.

The general course of the strands in the leaf base is at a slight angle upward from the point of connection with the cylinder; in very large leaves, the lower ones may run horizontally or even in a downward direction. Branching of some of the strands usually occurs within the cortex of the stem, so that as many as 20 strands may be found in the base of the petiole. This may not occur in small sterile leaves, so that a section of the petiole may show as few as 3 strands.

In the sterile leaves, the strands, 3–5 in number, arrange themselves in the shape of a C, with the opening directed adaxially. The strands in the extremities of the arc in passing up through the petiole swing out toward the margins as the blade is formed, and all come to lie in a single plane with xylem adaxially directed. Frequent branching and anastomosing of the strands occur, so that the number may reach 15 or 20 in the blade of the leaf.

In the fertile leaves, the strands, 4–12 in number, are arranged in a circle or oval as they leave the cylinder, and they maintain this arrangement through the petiole. They branch and anastomose freely, forming a complete cylindrical network; no strands connect across the circle. As the petiole broadens and flattens, the strands arrange themselves in two series: the outer, consisting of 10–15 strands with xylem directed adaxially, form the vascular system of the blade; the inner series, of 5–8 strands with xylem abaxially directed, is reduced by fusions to 4–6 strands which form the vascular supply of the spike. Anastomosing and branching occur as before within each series, but there is no further connection between the two systems. Beyond the point of connection of the fertile spike, the single series of strands constituting the reticulate veining of the blade may increase to as many as 30 in branching leaves. They form a closed system with the exception of a very few small branches which end blindly in the tip.

In the leaf strands as they separate from the cylinder, the xylem elements are all alike, but in the base of the petiole and throughout the leaf the first formed elements can be distinguished by their smaller size. These protoxylem elements are spiral vessels; they always occur at the inner margin of the xylem, that is, in the endarch position. The protophloem, consisting of 3 or 4 cells, develops at the opposite limit of the strand; the later developed phloem of 6–10 cells is arranged in 2 or 3 layers. One or two layers of parenchyma separate the xylem and phloem in a mature bundle (fig. 15).

In the mature strands of the blade, and, to a less extent, of the petiole, a definite bundle sheath is developed by the thickening of the walls of 2 or 3 layers of parenchyma surrounding the vascular elements (fig. 15). The thickening material is cellulose and the walls are pitted as in similar tissues of the stem and root. This bundle sheath is separated from the protoxylem by 2 or 3 cells of parenchyma, but borders directly upon the protophloem. As a consequence of growth within the bundle sheath, the protophloem in a mature strand is crushed against and between the cells of the sheath.

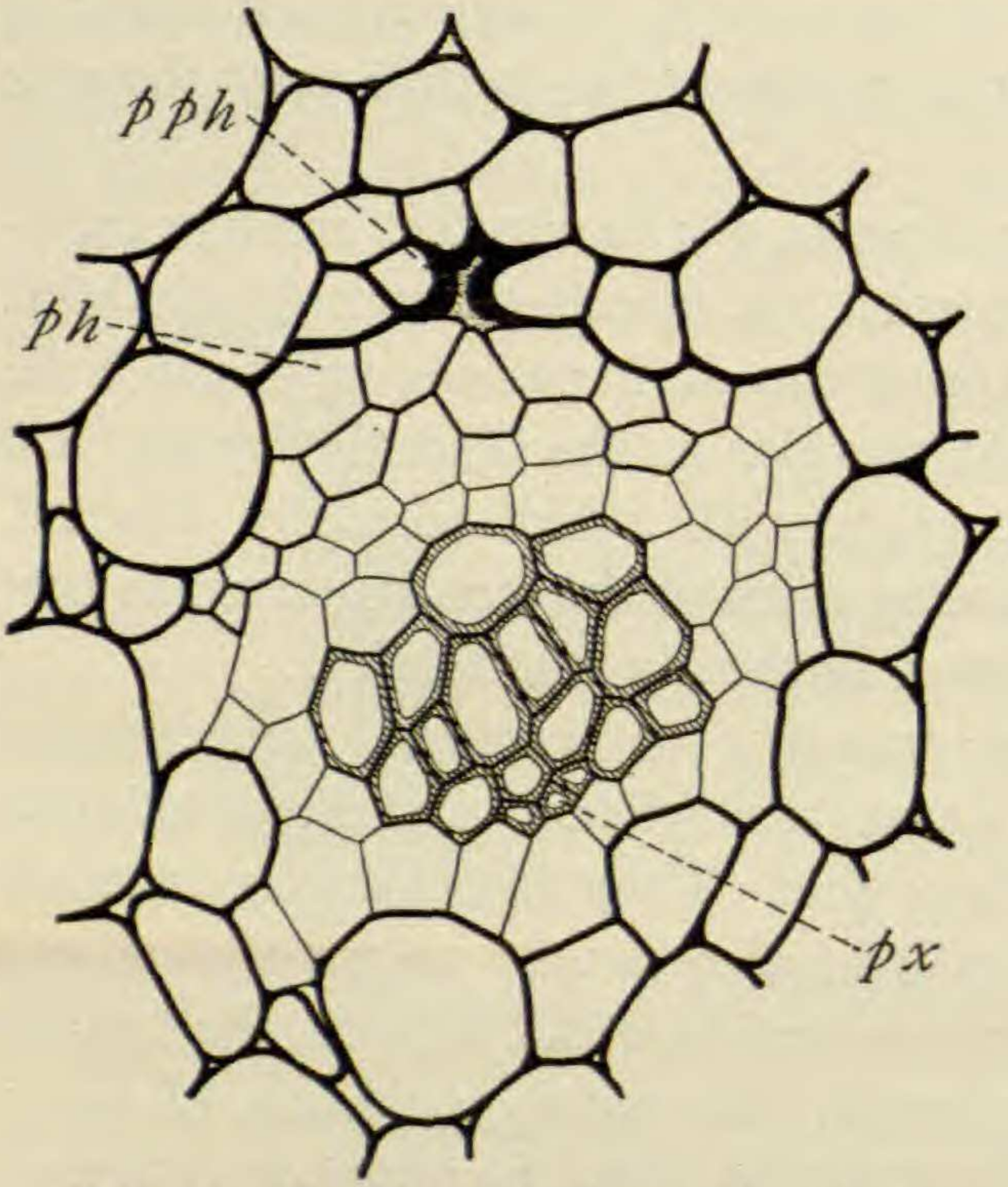

FIG. 15.—Detail of a leaf strand: *pph*, protophloem; *ph*, metaphloem; *px*, protoxylem; ×236.

The spike

As stated above, the strands of the leaf with xylem directed abaxially form the vascular supply of the spike. At the base of the peduncle these are 4–6 in number; they continue with occasional anastomosing and fusion through the median portion of the spike. At the base of the fertile portion of the spike, the strands at the margin of the median region run immediately at the base of the sporangia. Farther up the spike, where the ridge of sterile tissue which extends into the spore mass is well developed, the strand occupies a position well within this ridge (fig. 16, *B*). There is no branching of the marginal strand below the first few sporangia; but between the third and fourth sporangia, a short lateral branch extending halfway to the edge of the spike usually occurs. Similar strands occur between all the sporangia above this point (fig. 16, *A*). They consist in cross-section of 10 or 12 tracheids, and occupy the center of the thin wall separating adjacent sporangia. Near the margin of the spike they spread out in the shape of a fan and end blindly (fig. 16). The central strands are reduced by fusion to a single strand when the tip of the spike is reached. Between the terminal sporangia this splits into two strands which run out above the sporangia and end in the fan-shaped arrangement described above.

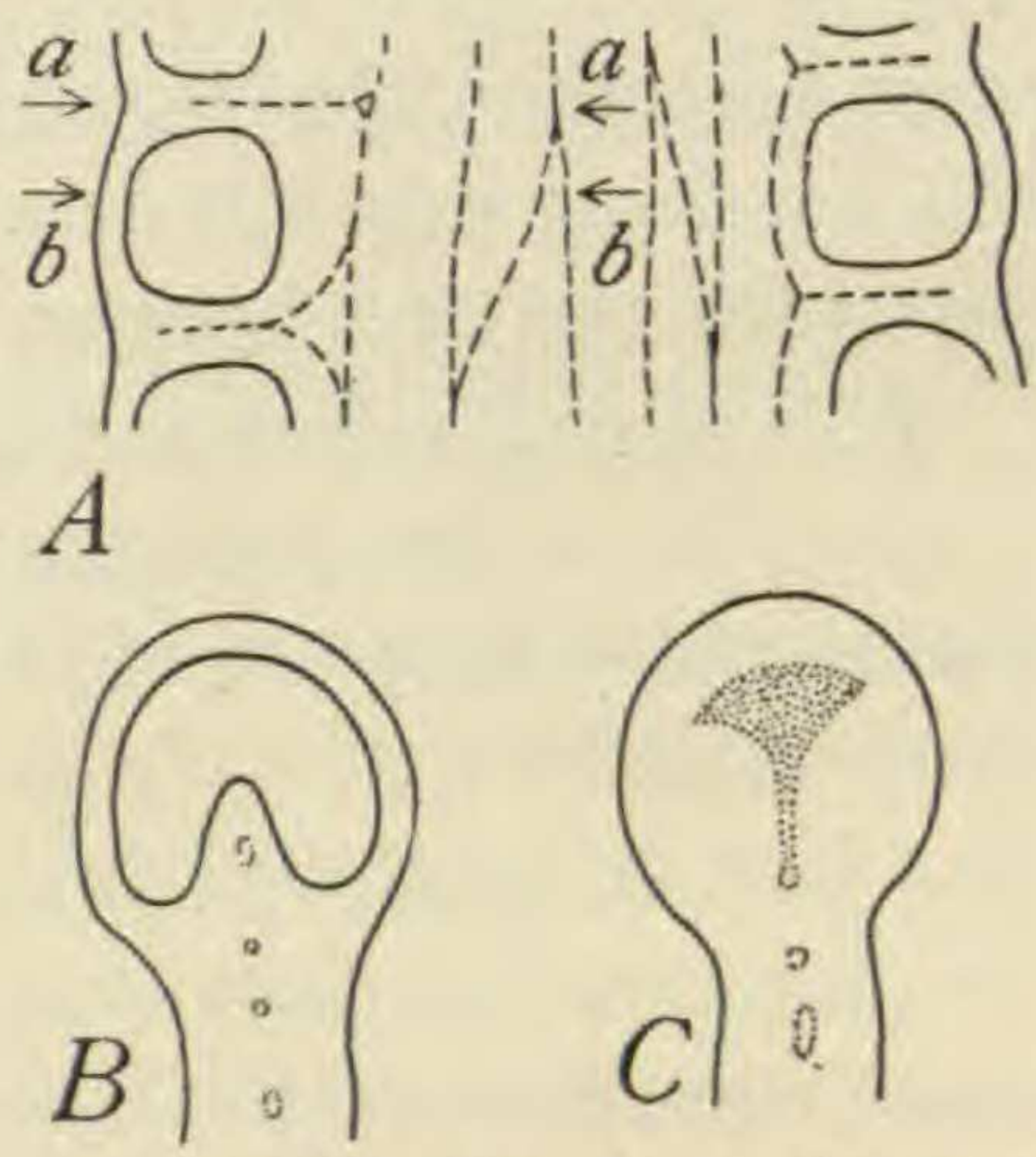

FIG. 16.—Diagrams of vascular system of the spike: *A*, median longitudinal section; *B*, transverse section in plane *bb*; *C*, transverse section in plane *aa*; ×4.5.

The strands of the peduncle and of the median region of the spike show the same structure as those of the blade of the leaf, but the bundle sheath is much less developed. In the strands between the sporangia, the bundle sheath is absent. The protophloem is on the adaxial side of the xylem, but the later developed phloem elements may extend in the shape of a U about the xylem, which is always endarch. In the fan-shaped portion of the strands the phloem cannot be distinguished; there is no distinction between protoxylem and metaxylem.

Discussion

The most striking characteristic of the anatomy of this species is the extreme variability of certain structures. The number of protoxylem strands of the root and the number of strands constituting the leaf trace vary almost directly with the size of the organs concerned. The external conditions which determine whether the stems are large in diameter or small determine in the same way the number of the strands of the leaf. Such variations may be considered as produced directly by growth conditions and therefore of physiological interest only.

The variability in the connection of the stem stele with that of the root is related to the position in which the bud develops; thus, the bud which developed the stem stele shown in fig. 7 was located directly over one of the protoxylem strands of the root, while that of the specimen shown in fig. 8 was placed midway between two such strands. In the same way, the condition represented in fig. 9 may be ascribed to a more gradual divergence of the root and stem axes; for a time, the two apical cells formed a common tissue within which a single stele was developed, as in fig. 10. When the angle between the two axes became greater, separate steles were developed.

It is of course impossible to say what determines these relations of position of the protoxylem strands and the stem meristem, or the rate of divergence of the two axes; but it seems evident from these variations that the manner of development of the stem stele in a bud is controlled by chance or external conditions. Hence we may conclude that the stelar characters shown in the development from buds cannot be used in any discussion of phylogeny. It is to be noted, however, that two features are constant; these are the collateral arrangement of the stelar elements and the endarch position of the protoxylem.

The occurrence of occasional strands of xylem in the pith is a feature that is unique, so far as the writer is aware. The manner of origin of these strands and their behavior suggests somewhat the medullary strands of *Marattia;* but the absence of phloem distinguishes sharply between the two cases. In any consideration of this feature it should be borne in mind that these strands occur to any considerable extent in only a single specimen.

Lang (5) from a study of *Botrychium Lunaria* has concluded that the pith of Ophioglossaceae is purely intrastelar in origin. This opinion is based in part upon the occurrence of scattered tracheids in the pith, particularly in injured rhizomes; this formation of xylem elements from parenchymatous cells of the pith is considered strong evidence of the stelar origin of the pith. The medullary strands of *O. pendulum* afford a much more definite case of this sort; they are in no way a traumatic response; cells of the pith develop a procambium which develops into xylem only; this may be taken to indicate that all cells of the pith are potentially xylem-producing, and therefore stelar.

Summary

1. The root stele varies from diarch to hexarch. The roots branch monopodially, and the branches are diarch at the base.

2. Buds develop upon the roots in the same manner as in *O. vulgatum*.

3. The rhizomes are always radial in structure; the leaves are inserted in an irregular spiral.

4. The connection of the stem stele with that of the parent root is very variable; no phylogenetic significance can be attached to the details of development of the stem stele of a bud.

5. The stele of a mature rhizome is an ectophloic siphonostele with large overlapping leaf gaps; there is no secondary thickening. Root gaps usually occur above the points of insertion of root strands. Incidental gaps not related to outgoing strands occur commonly.

6. In a single large rhizome, numerous xylem strands occur within the pith. These arise from the inner surface of the cylinder or as procambium; some of them are concerned in the closure of leaf gaps, and others disappear as procambium or by fusion with the cylinder. They consist of xylem only.

7. The vascular supply of the leaf consists of 3–12 strands, the number varying with the size of the leaf base. These strands form a cylindrical network in the petiole; in the lower portion of the blade, they constitute two series of strands with xylem oppositely directed. The strands with xylem abaxially directed form the vascular supply of the spike.

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